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Identifying the Mechanism(s) Responsible for the Translational Regulation of the Stress Signaling Kinase MKK4

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14. ABSTRACT
We recently demonstrated that the endogenous level of MKK4 is regulated post-transcriptionally in prostate cancer cell lines. Using WI-38 lung fibroblasts (WI-38 cells), which increase their MKK4 expression as they undergo senescence, we showed that a cohort of four miRNAs (miRs-15b, -24, -25, -141) decreased as the cells senesce. Treating WI-38 cells with precursor miRNAs (pre-miRs) to all four target miRNAs together but not individually, led to a significant decrease in MKK4 protein levels. Conversely, treatment with all four antisense miRNAs (AS-miRs) significantly increased MKK4 protein levels. To test if this effect is conserved in prostate cancer models, the levels of MKK4 mRNA and protein were quantitated in C4-2, CWR22-Rv1, DuPro, LAPC4, LNCaP, and PC3 cell lines and correlated to the endogenous levels of the cohort miRNAs. We hypothesized that miR-24 and miR-141 would be the best candidates in the cohort to regulate MKK4 protein expression based on the high versus low miRNA levels in the CaP lines and published data showing relevance to prostate cancer progression. However, our preliminary data showed that transfection of CWR22-Rv1 and PC3 cells with pre-miR-24 or pre-miR-141 did not decrease MKK4 protein levels. Moreover, transfection of those same lines with AS-miR-24 or AS-miR-141 did not increase MKK4 expression. Therefore the revised working hypothesis is that the entire cohort of miRs is required to affect the MKK4 protein levels. Current studies include the simultaneous transient transfection of all four miRs into CWR22-Rv1 and PC3 cells. Also, stable transfections of cohort Pre- and AS-miRNAs will allow us to test the effect of those miRs on the cells' metastatic potential.

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micro RNA, Prostate Cancer

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Introduction

When a primary tumor metastasizes to a secondary site, it has several barriers to overcome. Of those obstacles, the least understood is colonization, or formation of overt metastases from disseminated cancer cells. One tool in dissecting this process is mitogen-activated protein kinase kinase 4 (MKK4), a member of the stress-activated kinase family and a potent metastasis suppressor. Ectopic expression of MKK4 causes a significant reduction the number of spontaneous and experimental metastases and increases animal lifespan. Recently, our laboratory demonstrated that the endogenous level of MKK4 is regulated post-transcriptionally in prostate cancer cell lines. This prompted the hypothesis that the translation of MKK4 message is regulated, at least in part, by microRNAs (miRNAs). miRNAs are short (~22 nucleotide) single-stranded non-coding Using human diploid lung fibroblasts (WI-38 cells), which increase their MKK4 expression as they undergo senescence, we have previously demonstrated that the expression of a cohort of four miRNAs (miRs-15b, -24, -25, -141) decreased as the cells senesce. Treating WI-38 lung fibroblasts with precursor miRNAs premiRs) to all four target miRNAs together but not individually, led to a significant decrease in MKK4 protein levels. Conversely, treatment with all four antisense miRNAs (AS-miRs) significantly increased MKK4 protein levels (1).

Body

Using well-established prostate cancer cell line models, a series of studies are being conducted to determine whether miRNAs are involved in the translational repression of MKK4 protein in prostate cancer model systems. Working with our collaborators, the goal set forth in the statement of work for the first year of funding was to focus on the work in Study A of Specific Aim 1. In brief the goal of this work is to test the ability of candidate miRNAs to regulate MKK4 levels in prostate cancer cell lines. Preliminary studies in a model of fibroblast senescence found identified miR-15b, miR-25, miR-24, and miR-141 as regulators of MKK4 protein production (1).

We initially proposed to validate the differential expression of these miRNAs in prostate cancer cells lines with low MKK4 levels (i.e. PC3 and DuPro) and high MKK4 levels (i.e. LNCaP and C4-2). However, during the time that elapsed between submitting this proposal and its ultimate funding, other cell line models emerged as better choices for these studies. Thus, Mr. Robert Clark began his work on this project buy evaluating additional human prostate cancer cell lines (e.g. C4-2, CWR22-Rv1, and LAPC4) for their endogenous expression of MKK4 (Figure 1). We also quantitated the level of each of the candidate miRNAs in the cohort (Figure 2).

Using the approaches delineated in our proposal, we evaluated the relationship between levels of the MKK4 mRNA and protein in C4-2, CWR22-Rv1, DuPro, LAPC4, LNCaP, and PC3 cell lines and correlated to the endogenous levels of the cohort miRNAs. We hypothesized that miR-24 and miR-141 would be the best candidates in the cohort to regulate MKK4 protein expression based on the high versus low miRNA levels in the CaP lines and published data showing relevance to prostate cancer progression. However, our preliminary data showed that transfection of CWR22-Rv1 and PC3 cells with pre-miR-24 or pre-miR-141 did not decrease MKK4 protein levels (Figure 3a and 3c). Moreover, transfection of those same lines with AS-miR-24 or AS-miR-141 did not increase MKK4 expression (Figure 3b and 3d).

It should be noted that initiation of these studies was somewhat delayed due the time that it took to finalize the budget and assignment of personnel. As the PI I am delighted with the momentum that Mr. Clark and Dr. Thobe have now created. This bodes well for the progress of the work in year two.

Key Research Accomplishments

Optimization and improvement in the choice of prostate cancer cell lines/model systems being used in these studies.

Evaluating the relationship between specific miR and MKK4 levels in each cell line.

The finding that modulating miR-24 or miR-141 singly is not likely to affect MKK4 levels in the prostate cancer cell lines tested.

Reportable Outcomes

The regulation of mitogen-activated kinase kinase 4 by a microRNAs in prostate cancer. Robert Clark, Kristen Otto, Bernard Marasa, Myriam Gorospe, Carrie Rinker-Schaeffer Abstract accepted for poster presentation at the Metastasis and the Tumor Microenvironment – Joint Meeting of the Metastasis Research Society and the AACR September 12-15, 2010

The regulation of mitogen-activated kinase kinase 4 by a microRNA cohort in prostate cancer cell lines. Robert Clark, Megan Thobe, Kristen Otto, Bernard Marasa, Myriam Gorospe, Carrie Rinker-Schaeffer Abstract submitted for the 2011 IMPaCT Conference

Conclusion

Our data have prompted us to revise our working hypothesis. Thus, we currently hypothesize that, as in the fibroblast senescence model, the entire cohort of four miRs is required to affect the MKK4 protein levels. Current studies include the simultaneous transient transfection of all four miRs into CWR22-Rv1 and PC3 cells. Also, stable transfections of cohort Pre- and AS-miRNAs will allow us to test the effect of those miRs on the cells' metastatic potential. These studies are designed to delineate an important and functional role for miRNAs in regulating MKK4 levels and hence its metastasis suppressor function in prostate cancer.

References

1. Marasa BS, Srikantan S, Masuda K, Abdelmohsen K, Kuwano Y, Yang X, Martindale JL, Rinker-Schaeffer CW, Gorospe M. Increased MKK4 abundance with replicative senescence is linked to the joint reduction of multiple microRNAs. Sci Signal. 2009 Oct 27;2(94):ra69.

Appendices

Figure 1, Figure 2, Figure 3.

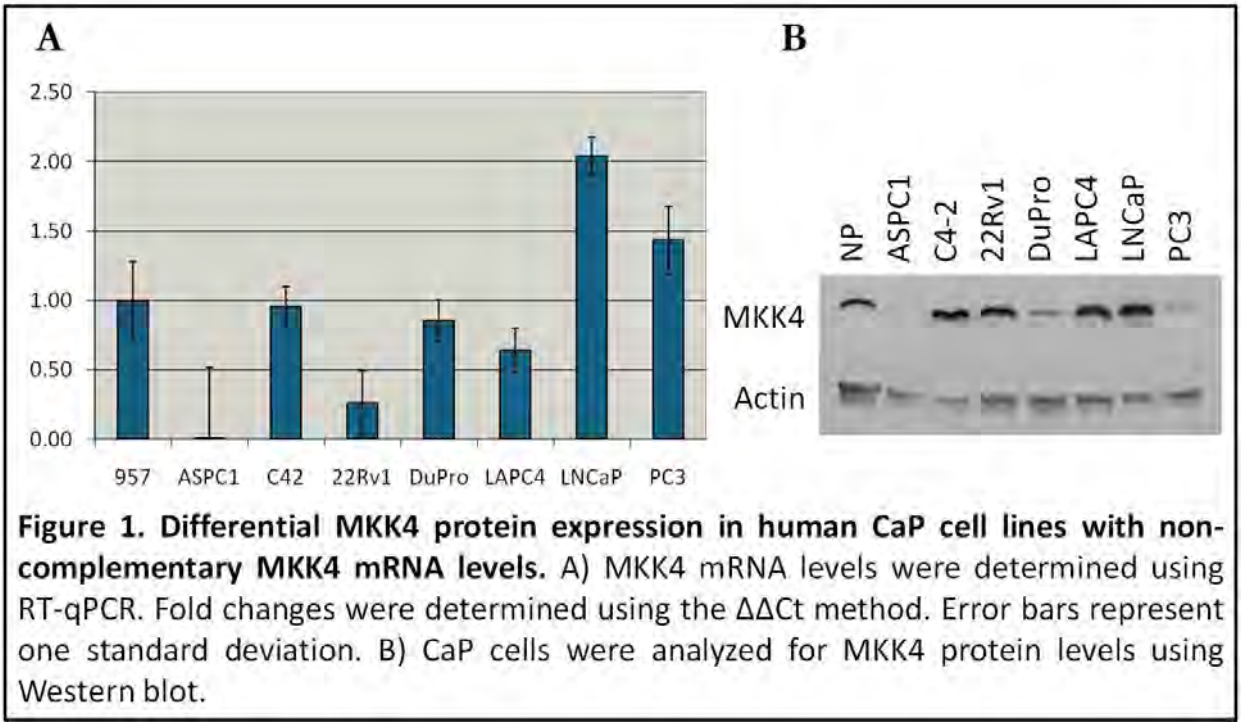

Figure 1. Differential MKK4 protein expression in human CaP cell lines with non-complementary MKK4 mRNA levels. A) MKK4 mRNA levels were determined using RT-qPCR. Fold changes were determined using the ΔΔCt method. Error bars represent one standard deviation. B) CaP cells were analyzed for MKK4 protein levels using Western blot.

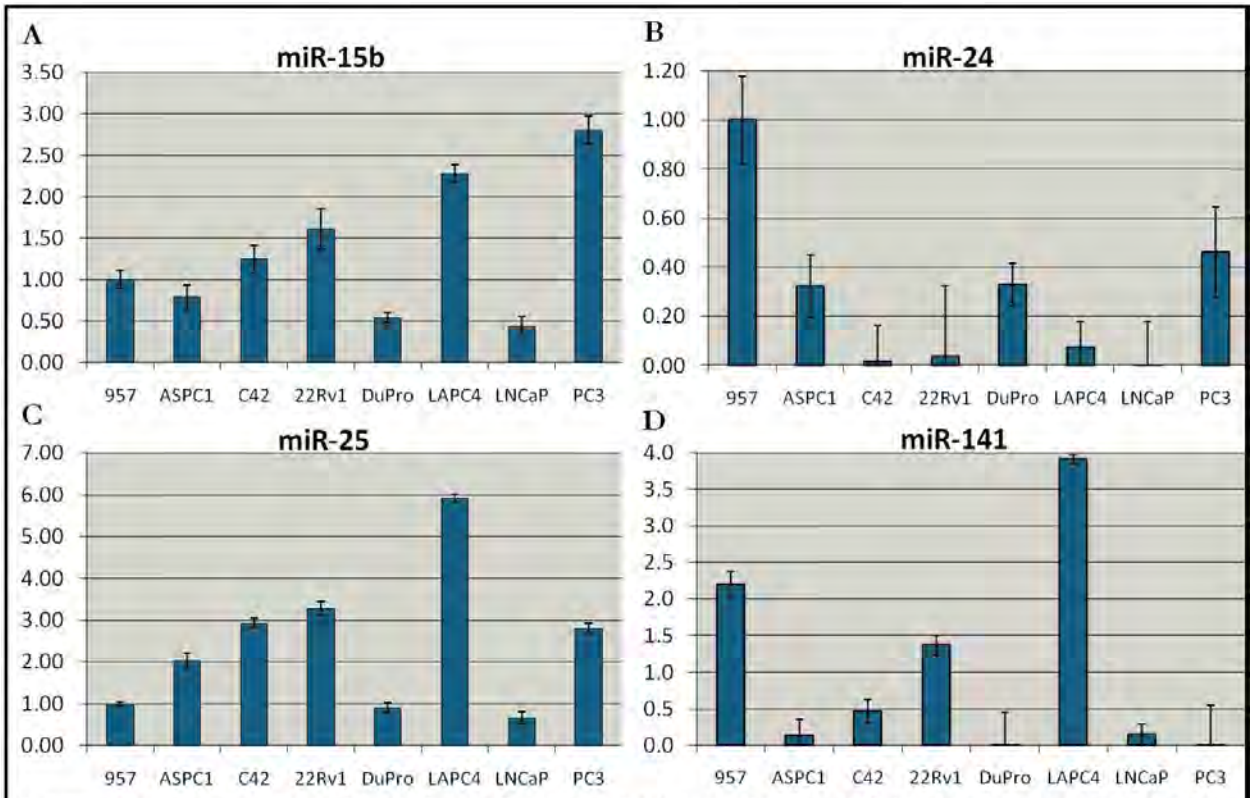

Figure 2. Endogenous levels of the individual miRNAs in the potentially MKK4 regulating cohort. MicroRNA levels were assayed using RT-qPCR in proliferating cells. A) miR-15b; B) miR-24; C) miR-25; D) miR-141. Fold changes were determined using the ΔΔCt method. Error bars represent one standard deviation.

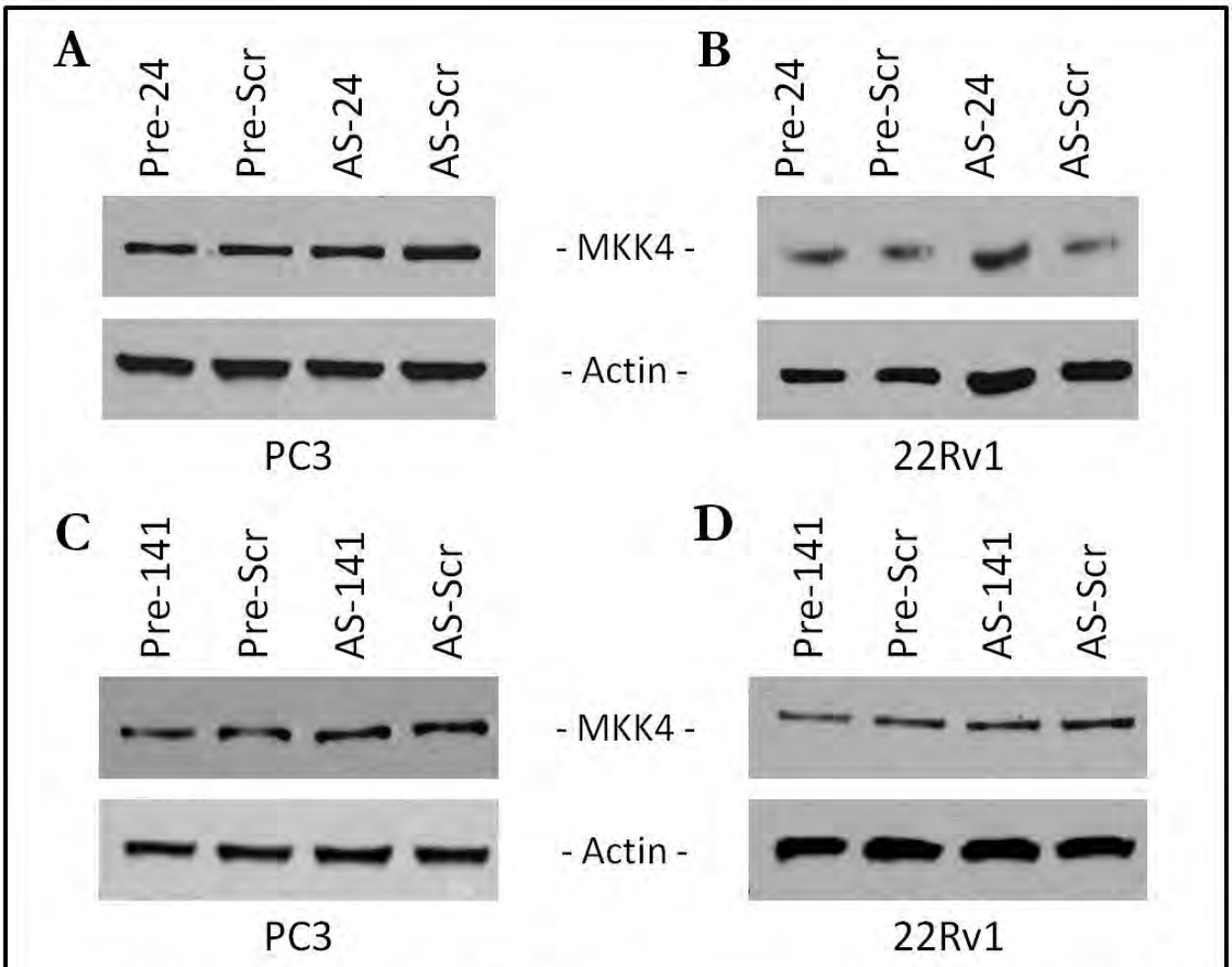

Figure 3. MicroRNAs-24 and -141 do not regulate MKK4 protein levels in PC3 and CWR22-Rv1 cells. CaP cells were transiently transfected with Pre-miR-24 and AS-miR-24 (A-B) or Pre-miR-141 and AS-miR-141 (C-D) using a reverse transfection protocol. Scrambled (Scr) controls were used.